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Supplementary Materials for

IL-27 signaling activates skin cells to induce innate antiviral proteins and protects against Zika virus infection

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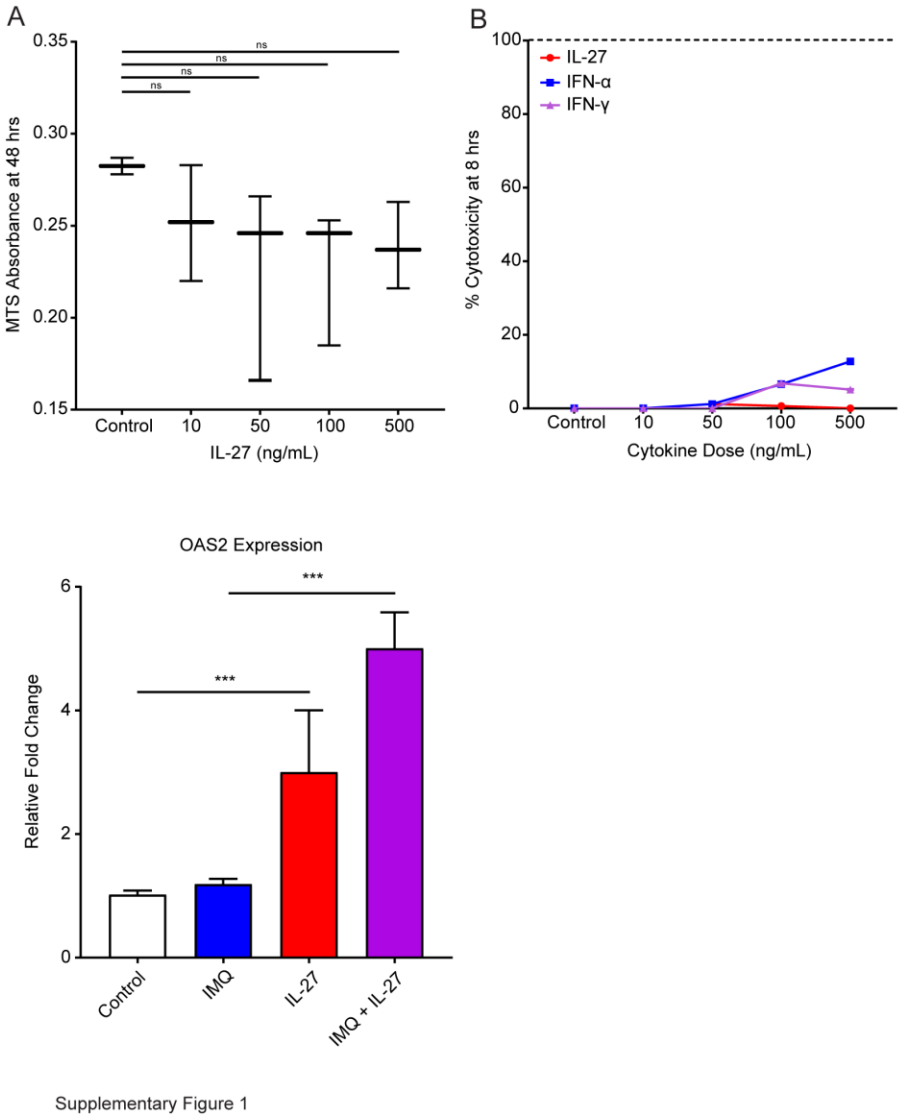


Supplementary Figure 1

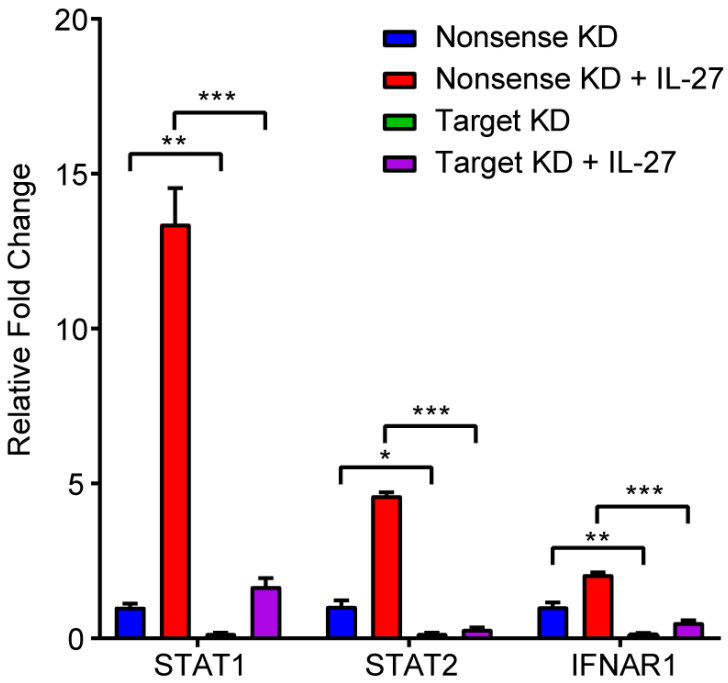
Supplementary Figure 1. IL-27 Treatment of Human Keratinocytes is not associated with Cytotoxicity

(A) MTS absorbance at 48 hours of human keratinocytes treated with IL-27 at the indicated concentrations. The MTS absorbance at each indicated concentration of IL-27 was compared to the non-treated control. n = 3 biological replicates.

(B) % cytotoxicity measured as a percent of LDH absorbance in human keratinocytes treated with IL-27, IFN-α, and IFN-γ at the indicated concentrations for 8 hours. The %

cytotoxicity at 8 hours for IL-27, IFN-α, and IFN-γ was compared at each cytokine dose. n = 3 biological replicates.

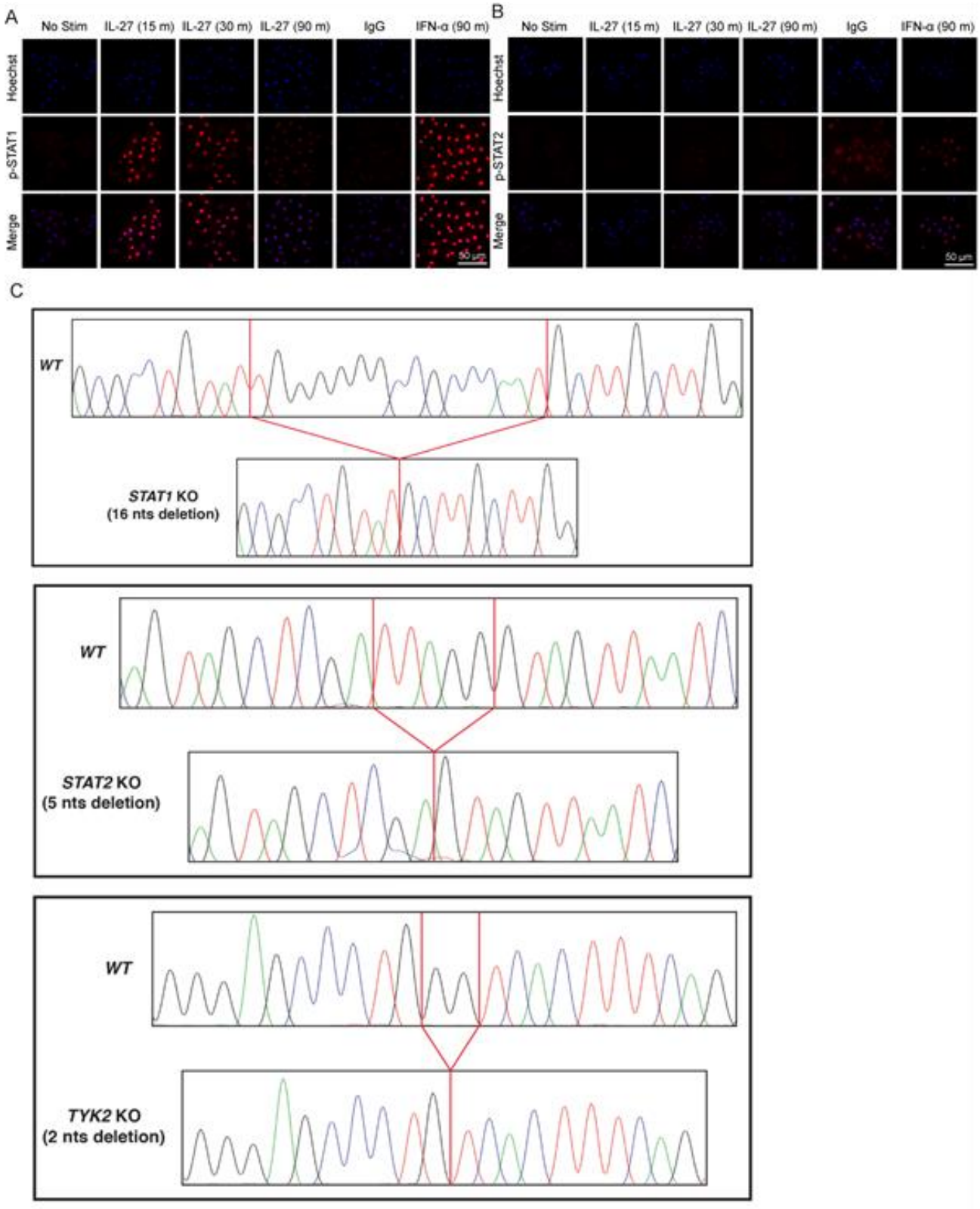
(C) qPCR analysis examining the expression of *OAS2* by primary human keratinocytes treated with IMQ and/or IL-27 (100ng/ml) for 24 hours. Relative fold change of *OAS2* for each condition was compared to the non-treated control. *n* = 3 biological replicates. To test for statistical significance, a two-tailed Student's unpaired t test (A and C) was performed using GraphPad Prism software. Data are mean $\pm$ SEM. *p < 0.05, **p < 0.01, ***p < 0.001.



Supplementary Figure 2

Supplementary Figure 2. Confirmation of Successful siRNA Knockdown

qPCR analysis of expression of STAT1, STAT2, and IFNAR1 in response to siRNA transfection with the corresponding siRNA transcripts. The relative fold change of IL-27 treated cells was compared to their non-IL-27-treated controls within both nonsense KD and target KD treated groups. n = 3 biological replicates. To test for statistical significance, a two-tailed Student's unpaired t test was performed using GraphPad Prism software. Data are mean $\pm$ SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.



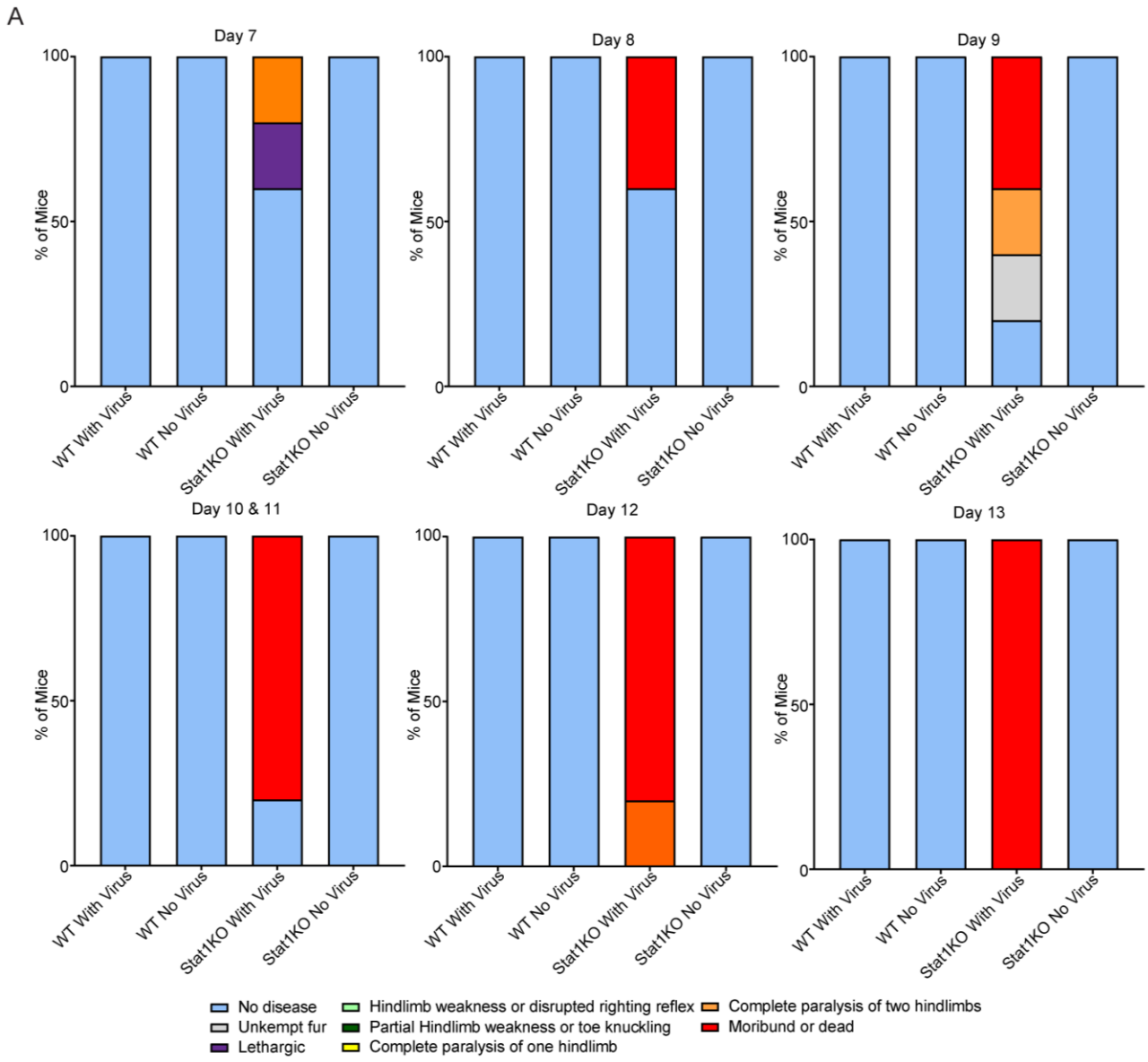
Supplementary Figure 3

Supplementary Figure 3. IL-27 Signaling Results in Rapid and Transient Nuclear Translocation of STAT1, but not STAT2, and Sanger Sequences for STAT1, STAT2, and TYK2 CRISPR-Cas9 KO Keratinocytes

(A) Immunofluorescence staining for p-STAT1 (Cy3) in primary human keratinocytes treated with a time course of 100 ng/mL of IL-27 of 50 U/mL of IFN-α. n = 3 biological replicates.

(B) Immunofluorescence staining for p-STAT2 (Cy3) in primary human keratinocytes treated with a time course of 100 ng/mL of IL-27 of 50 U/mL of IFN-α. n = 3 biological replicates.

(C) Sanger sequence of *STAT1, STAT2,* and *TYK2* KO CRISPR-Cas9 KO keratinocytes.

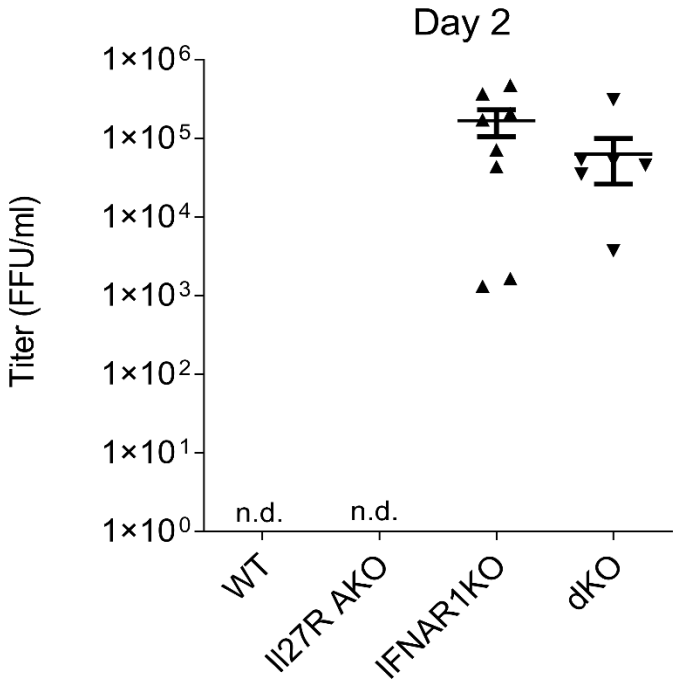


Supplementary Figure 4

Supplementary Figure 4. *Stat1* Signaling is Required for Antiviral Defense Against Zika Virus

(A) Bar graphs detailing morbidity and mortality of 129S6 (WT), and *Stat1*KO mice injected 10^4 Zika virus (HPF2013 strain) at 5-10 days post-infection. Symptoms were scored in a sequential fashion, where lesser symptoms (e.g. hunched, unkempt fur) were not counted if a mouse presented with more severe symptoms (e.g. hindleg

weakness). n = 4 mice (129S6) and 5 mice (*Stat1*KO) per experimental group. Experiments was repeated 3 times.



Supplementary Figure 5.

Supplementary Figure 5. Focus forming assay of sera from Zika virus-infected mice 2 days post-infection. Titers were calculated as FFU/mL and graphed as percent of Ctrl (Control: No cytokine treatment). To test for statistical significance, a two-tailed Student's unpaired t test was performed using GraphPad Prism software and no statistical significance between Ifnar1KO and dKO was observed.

SUPPLEMENTARY Materials

KEY RESOURCES TABLE S1

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit polyclonal anti-OAS2	Proteintech	Cat #: 19279-1-AP
Rabbit polyclonal anti-MX1	Abcam	Cat # AB 95926
Rabbit polyclonal pSTAT1	Cell Signaling Technologies	Cat #: 9167S
Rabbit polyclonal pSTAT2	Cell Signaling Technologies	Cat #: 88410S
Rabbit polyclonal anti-IRF3	Cell Signaling Technologies	Cat #: 11904T
Virus Strains		
ZIKV-Dakar (DAK; Zika virus/A.africanus-tc/SEN/1984/41525-DAK)	University of Texas Medical Branch	GenBank accession #KU955591
ZIKV-French Polynesia (PF; Zika virus strain H/PF/2013	University of Texas Medical Branch	GenBank accession #KJ776791.2
Chemicals, Peptides, and Recombinant Proteins		
Human IL-6	Biolegend	Cat #: 570802
Human IL-12 p70	Tonbo	Cat #: 21-8129-U010
Human IL-23	Biolegend	Cat #: 574102
Human IL-27	Biolegend	Cat #: 589204
Human IL-35	Biolegend	Cat #: 578502
Human IFN-α2	Biolegend	Cat #: 592704
Human IFN-γ	Peprotech	Cat #: AF-300-02
Mouse IL-27	Biolegend	Cat #: 577406
Experimental Models: Cell Lines		

Human epidermal keratinocytes (adult)	Thermo Fisher	Cat #: C0055C
Experimental Models: Organisms/Strains		
C57Bl6/J mice	Jackson Labs	Stock No: 000664
Il27ra$^{-/-}$ mice	Jackson Labs	Stock No: 018078
Ifnar1$^{-/-}$ mice	Jackson Labs	Stock No: 32045-JAX
BALB/c mice	Jackson Labs	Stock No: 000651
Stat1$^{-/-}$ mice	Taconic	Model No: 2045
129S6 mice	Taconic	Model No: 129SVE
Ifnar1$^{-/-}$/Il27ra$^{-/-}$ double KO mice	Bred Locally	Available upon request
Oligonucleotides		
Human OAS1 Primer, Forward: GTCTTCCTCAGTCCTCTCACG, Reverse: AAGGCAGGCAGCACATCG	This Paper	N/A
Human OAS2 Primer, Forward: TCAGAAGAGAAGCCAACGTGA, Reverse: CGGAGACAGCGAGGGTAAAT	This Paper	N/A
Human OAS3 Primer, Forward: ACTGACATCCCAGACGATGC, Reverse: CCAGTGCTCACCTAGGGC	This Paper	N/A
Human OASL Primer, Forward: CGTGAAACATCGGCCAACTAAG, Reverse: GTACCCATTTCCCAGGCATAGA	This Paper	N/A
Human MX1 Primer, Forward: GGGGAGGAAATAGAGAAAATGAT, Reverse: GTTTACAAAGGGCTTGCTTGCT	This Paper	N/A
Mouse Oas1a Primer, Forward: GGAGGCGGTTGGCTGAAGAGG, Reverse: GAACCACCGTCGGCACATCC	This Paper	N/A
Mouse, Oas2 Primer, Forward: CCGGGCCAGTGCACAAGTTAG, Reverse: CGATGGCACCGAGGACACC	This Paper	N/A

Mouse, Oas3 Primer, Forward: TCTGGGGTCGCTAAACATCAC, Reverse: GATGACGAGTTCGACATCGGT	This Paper	N/A
Mouse Oasl1 Primer, Forward: CCAGGAAGAAGCCAAGCACCATC, Reverse: AGGTTACTGAGCCCAAGGTCCATC	This Paper	N/A
Mouse Oasl2 Primer, Forward: TTGTGCGGAGGATCAGGTACT, Reverse: TGATGGTGTCGCAGTCTTTGA	This Paper	N/A
See Table S1 for mouse Oasl2 primer, human STAT1 primer, human STAT2 primer, human IFNAR1 primer	This Paper	N/A
Software and Algorithms		
Human Affymetrix microarray data	GSE143228	N/A
Other code available upon request	David Corcoran	N/A